[3] Y.C. Sasaki et al., Phys. Rev. E 62:3843 (2000)
[4] H. Sekiguchi et al, PLOS ONE 8:e64176 (2013)
[5] H. Sekiguchi et al, Scientific Reports 4:6384 (2014)

963-Plat
An ELIC-GLIC Chimera Reveals Distinct Pathways of Activation in the Cys-Loop Family of Receptors
Nicolaus Schmandt, David T. Lodowski, Vivien Yee, Sudha Chakrapani.
Case Western Reserve University, Cleveland, OH, USA.
The Cys-loop family of pentameric ligand-gated ion channels is expressed throughout the human brain and is a target for a wide number of therapeutics including local and general anesthetics, alcohols, and neurosteroids. Recently, high-resolution structures of several members of the family have become available, but allosteric mechanisms governing gating and modulation remain unclear. Prokaryotic homologues (ELIC and GLIC) have the same overall structure as the family of ion channels, and are highly amenable to structural studies in a native environment. We investigated the conformational dynamics of ligand-binding and channel gating in GLIC and ELIC by electron paramagnetic spectroscopy (EPR) and found that the two channels exhibit distinct activation mechanisms. To further understand how the two events are coupled, we engineered a functional chimera that included the extracellular domain of ELIC and the transmembrane domain of GLIC. By using a combination of electrophysiology, EPR spectroscopy, and X-ray crystallography we show the molecular details of coupling between the ligand-binding domain and the channel gate.

964-Plat
Disulfide Trapping the GABA-A Receptor Extracellular Beta-5/Beta-5' Loop
Cassandra M. Theusch, Cynthia Czajkowski.
Neuroscience, University of Wisconsin - Madison, Madison, WI, USA.
$GABA_A$ receptors (GABARs) couple GABA binding to opening of a chloride-conducting channel. The GABA binding site is at β/α-subunit interfaces. In a recent GABAR crystal structure, the β5-β5' loop reaches across the interface, and juts into the neighboring subunit forming a back lid over the GABA binding site. This extended β5-β5' loop conformation is not in prokaryotic channels, the acetylcholine-binding protein, or the nicotinic acetylcholine receptor. We used disulfide trapping to probe β5-β5' loop position and dynamics in $\alpha_1\beta_2\gamma_{2L}$ GABARs. We engineered pairs of cysteines at the β/α GABA-binding site interface: αM111C (β5-β5' loop) with βS104C (β-strand 5) and α_1M113C (β5-β5' loop) with β_2D95C (binding Region A). We expressed single and double mutant $\alpha_1\beta_2\gamma_{2L}$ GABARs in *Xenopus* oocytes. Initially, we tested for disulfide-bonds by measuring effects of DTT and hydrogen peroxide on GABA-mediated currents from α_1M111Cβ_2S104Cγ_2 and α_1M113Cβ_2D95Cγ_2 receptors. They had no effects. Methanethiosulfonate reagents modified the single cysteine mutants but not the double mutants, indicating the cysteine pairs were linked by disulfides. Single-mutant $\alpha_1\beta_2$S104Cγ_2 receptors had reduced GABA efficacy and potency, which were rescued in double-mutant α_1M111Cβ_2S104Cγ_2 receptors, suggesting the cysteines interact. These results indicate the α-subunit β5-β5' loop extends across the intersubunit interface, near β-strand 5 of the β-subunit. Disulfide linking these two regions had no effect on GABA currents, indicating their relative positions do not change during GABA activation and supporting the idea of a concerted GABA-driven motion in the inner β-sheets of the α- and β-subunits. Linking cysteines in aligned positions at the benzodiazepine-binding α_1/γ_2-interface (α_1S106C and γ_2I124C) had similar effects. In contrast, tethering α_1M113C (β5-β5' loop) to β_2D95C near Region A of the GABA binding site right-shifted GABA concentration-responses, suggesting this crosslink impairs motions in the site required for GABA action.

965-Plat
Conformational Dynamics in the $GABA_A$ Receptor
Rilei Yu, **Philip C. Biggin**.
Biochemistry, Oxford University, Oxford, United Kingdom.
$GABA_A$ receptors are members of the cys-loop family of ligand-gated ion channels and are pentameric ion channels that are closed in the absence of ligand, but upon ligand-binding, open to allow anions to pass through a central pore. The sustained application of agonist however leads to the receptor entering a desensitized state whereby the agonist is still bound but the channel is closed. In order for the channel to fully respond to agonist again, agonist must diffuse away and the receptor return from the desensitized state to the resting state. The recent crystal structure of the human β3 $GABA_A$ receptor was proposed to be locked in a desensitized state due in part to the presence of agonist in the crystallization conditions and the apparent closed diameter of the pore [1]. Key questions still remain about how these receptors move between the different conformational states. Here we use MD simulations to explore potential mechanisms of conformation change. Removal of the benzamidine from the binding site should favour a transition from the desensitized state towards the resting state. In the simulations, removal of agonist reveals that the pore-lining TM2 helices are able to change conformational and adopt orientations that may be more consistent with a resting rather than desensitized state of the channel. In this work, we discuss the apparent coupling between the binding site, the β1β2 linker and the TM2-TM3 loop during these changes and the implications for how cys-loop receptors in general cycle through distinct conformational states. We also discuss the behavior of a key tyrosine (299) proposed to play a role in stabilizing the desensitized state.
References:
1. Miller PS, Aricescu AR. Crystal structure of a human $GABA_A$ receptor. Nature. 2014 512:270-5.

966-Plat
Monitoring the Work of a Single Subunit in Homotetrameric CNGA2 Channels
Klaus Benndorf[1], Nisa Wongsamitkul[1], Vasilica Nache[1], Thomas Eick[1], Sabine Hummert[1], Eckhard Schulz[2], Ralf Schmauder[1], Jana Schirmeyer[1], Thomas Zimmer[1].
[1]Institute of Physiology II, Friedrich-Schiller-University, Jena, Germany, [2]Faculty of Electrical Engineering, University of Applied Sciences, Schmalkalden, Germany.
Cyclic nucleotide-gated (CNG) channels mediate signal transduction in photoreceptors and olfactory cells. Wild-type olfactory CNG channels are composed of three types of subunits, CNGA2, CNGA4, and CNGB1b. Out of these only CNGA2 subunits can form functional homotetrameric channels when expressed in heterologous systems. Homotetrameric CNGA2 channels are very useful for studying elementary biophysical processes. However, even for homotetrameric channels the transmission of the ligand binding to the pore opening is still a mystery. Undoubtedly clear is only that the activation of the channels is not generated by independently operating but by interacting subunits. We are interested in how a single subunit embedded in a channel binds a ligand and evokes channel activation, i.e. for the whole channel we intend to kinetically dissect the molecular gating mechanism induced by the first ligand binding step. We constructed tetrameric concatamers of CNGA2 channels with various numbers of wild-type and mutated binding domains of high and extremely low affinity. Ligand binding was measured by confocal patch-clamp fluorometry (frame rate up to 277 images per second) using a fluorescent cGMP analogue. Our results show that a single subunit operates a channel only at 23-fold higher ligand concentrations than a tetrameric channel composed of four cooperating subunits. Nevertheless, the single subunit opens the channel pore to the full conductance level. Moreover, we show that Markovian models with a discrete number of states surprisingly fail to describe the action of the only wild-type subunit. Instead, a model consisting of three separate but intimately coupled processes proved to be adequate for describing channel activation, containing a rapid ligand binding close to the diffusion limit, a continuous conformational diffusion upon gating consisting of two components, and a rapid and discrete pore action.

967-Plat
Allosteric Regulation of the Cyclic Nucleotide-Binding Domain in HCN Channels
Hannah A. DeBerg[1,2], Shahidul M. Islam[3,4], Michael C. Puljung[2], Benoit Roux[3,4], William N. Zagotta[2], Stefan Stoll[1].
[1]Chemistry, University of Washington, Seattle, WA, USA, [2]Physiology and Biophysics, University of Washington, Seattle, WA, USA, [3]Biochemistry and Molecular Biology, University of Chicago, Chicago, IL, USA, [4]Gordon Center for Integrative Science, University of Chicago, Chicago, IL, USA.
Hyperpolarization-activated cyclic nucleotide-gated (HCN) ion channels play an important role in regulating pacemaking activity in the heart and brain. They are regulated by the binding of cyclic nucleotides to a conserved, intracellular cyclic nucleotide-binding domain (CNBD). Binding of cyclic nucleotides increases the rate and extent of activation of the channels and shifts channel activation to less hyperpolarized voltages. In intact channels, cAMP and cGMP are full agonists and cCMP is a partial agonist. We use double electron-electron resonance (DEER) to study the conformational change associated with the binding of these three different cyclic nucleotide species to the CNBD. We find that conformational changes associated with ligand binding vary depending on the cyclic nucleotide bound and the location in the CNBD. We use the restrained ensemble MD (re-MD) simulations method to generate structural models integrating the complete set of experimentally measured data from DEER distance distribution histograms that describe the separation between pairs of spin labels attached to the CNBD. Our results indicate that, in the B-helix, binding of cGMP or cCMP has an